Ecological and Chemical Analysis of Heavy Metal Transduction in *Salix exigua* on the Animas and Florida Rivers

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Abstract

On August 5th, 2015, an accident at the Gold King Mine in Silverton, Colorado triggered the release of three million gallons of heavy metals into the Animas River. As heavy metals have toxic effects in high concentrations over time, it is extremely important to quantify the amounts of heavy metals in both the water itself, as well as surrounding riparian zones. This study inquired as to whether heavy metals were present in *Salix exigua*, or coyote willow, which makes up a large portion of the riparian biota. Samples were taken from three sites, at Oxbow Park and Preserve and Trimble Lane on the Animas River, as well as from a control site on the Florida River. Six metals, including aluminum, zinc, cadmium, manganese, barium, and iron, were quantified in root and leaf samples to account for the fate and transport into riparian plants. As bioaccumulation of metals in ecosystems can have effects in many areas, assessing the concentrations in the flora surrounding the river is essential to accounting for all aspects of river health. Metals were found to be significantly higher in roots compared to shoots, across all sites. Furthermore, the Animas River had significantly higher concentrations of heavy metals than the control site. Specifically, Oxbow Park and Preserve had the highest levels due to the geomorphology of this river reach. This study was essential for the quantification of heavy metal concentrations in the Animas River, and will help gain insight to the ecological health. It may also serve as baseline data for future studies accounting for plant health in this area.

Introduction

Acid mine drainage and the effects of heavy metals within both aquatic and riparian ecosystems are coming to the attention of scientists and communities all over the United States. With the high frequency of mine spills and acid mine drainage, it is essential that we understand the short and long-term effects of heavy metals in river systems. When oxygenated water comes into contact with mine tailings containing iron sulfide, the chemical reaction causes the formation of sulfuric acid, which contaminates the water. Sulfuric acid both increases the acidity of the water and leads to other heavy metals entering the solution in either a dissolved or colloidal form that would not have been present otherwise (Cutright et al., 2012). While dissolved forms of heavy metals cause a more negative impact than the precipitate form, the abundance of heavy metals such as iron, arsenic, cadmium, lead, and aluminum are all of serious environmental concern regardless of their fate and transport mechanisms and represent a great challenge with regards to acid mine drainage (Gray, 1997).

In light of the recent 2015 Gold King mine spill that washed three million gallons of water containing heavy metals into the Animas River, there is no better time to investigate the effects of heavy metal pollutants on this river and its underlying ecology (USDI, 2015). By looking at heavy metal uptake in plants within multiple zones and locations of the river, we can identify what areas of the Animas River have been most affected by the spill and quantify observable differences in terms of stream gradient and morphology.

Current Status of Mines and Remediation Efforts

Acid mine drainage is a major cause of river pollution in many countries with both historic and current mining practices. The preferred treatment option is preventing the formation and migration of acid mine drainage from its source; however, this is not always a feasible method. If prevention is possible, this generally involves either flooding and sealing of mines, land-based storage of mine tailings, or underwater storage of mine tailings (Johnson & Halberg, 2005). If prevention is not a feasible method, the next step is some form of remediation, usually involving a biological or chemical mechanism to neutralize acid mine drainage and remove heavy metals out of solution and into a solid, less harmful form. Remediation can use abiotic or biotic methods, but most commonly involves the addition of a chemical-neutralizing agent (abiotic). Alkaline material added to an acidic environment acts as a buffer and accelerates the rate of metal oxidation, thereby balancing the pH of the river. This helps precipitate heavy metals out of solution, so they are not in their dissolved form and are less dangerous to both humans and the environment as a whole. Disadvantages to these techniques are that they are expensive, ineffective, and typically create a toxic chemical sludge that is difficult to remove from the ecosystem(Johnson & Halberg, 2005; Ahluwalia & Goyal, 2007).

Effects of Acid Mine Drainage on Ecosystems and Vegetation

As acid mine drainage gains more attention as a large environmental issue, the effects on ecosystems are being studied in more depth. Major impact areas include lakes, estuaries, coastal waters, and the particular focus of this study: rivers. The effects of acid mine drainage are sorted into four categories: metal toxicity, sedimentation, acidity, and salinization. While all are important, most studies focus on metal toxicity as it poses the most direct effects on ecosystem health and resiliency. Some of the most detrimental effects of heavy metal toxicity on an ecosystem generally include habitat modification, niche loss, bioaccumulation within a food chain, losses of food source, and ultimately elimination of sensitive species (Gray, 1997). The impact of acid mine drainage is difficult to quantify and predict, particularly in continuously flowing waters such as rivers. In response, the US Agency for Toxic Substances and Disease Registry lists lead, mercury, and arsenic as the top first, second, and third most hazardous metals, respectively. As heavy metals bioaccumulate in smaller organisms such as macroinvertebrates and plants, higher organisms in the food chain such as birds, herbivores, and even humans will start to experience biomagnification of these heavy metals as well (Rai, 2008).

Heavy metals are not simply stored in the sediment of rivers, nor do they merely continue flowing forever through the water. Plants that are present within a riparian zone experience bioaccumulation of heavy metals through root contact with sediment, soil, and water over time. This is an important concept to highlight, as not only aquatic organisms experience effects, but many organisms within the riparian zones as well (Tangahu et al., 2011). One recent study correlated increased levels of certain heavy metals, such as zinc, copper, and lead, to a decrease in root biomass in the genus *Brassica.* Other general effects that heavy metals have on plants include a decreased rate of photosynthesis, decrease in overall water content, and stunted growth of an entire plant, especially within the roots (Mourato et al., 2015). As vegetation is an important source of food, shelter, and detritus in a riparian and aquatic ecosystem, if plants are being affected by heavy metal uptake, this will reflect upon many organisms within an ecosystem. Many plants transduce heavy metals from their roots into their shoots and leaves, which is important in understanding the ways in which acid mine drainage moves out of the water and into the riparian zone (Cutright et al., 2012).

The amount of heavy metals present within an aquatic or riparian plant is not solely determined by the amount of that metal in the water, sediment, or soil. The limiting factor, instead, is the metal tolerance of the plants themselves. Plants have different mechanisms and genes that allow for increased solubility of metals into the roots. Furthermore, some riparian plants have transport proteins that move metals into root cells, and eventually the vascular system and ultimately leaf cells. This diversity within plant genetics explains why many plants may have high levels of a particular metal and the ability to translocate these metals into shoots and leaves, while other plants cannot tolerate high levels whatsoever. For example, pine pennycress (*Thlaspi)* thrives on soils with high levels of cadmium and zinc. After observing the molecular physiology, researchers found that key gene sites for zinc transport were very stimulated in this plant in comparison with other species (Becker, 2000).

Knowing that plants have different tolerances and mechanisms for taking up metals can help increase our understanding of variation within the community and assess the overall health of the ecosystem.

Salix spp. and Heavy Metal Transduction

Because different species have varying abilities in both uptake and transduction of metals, it would be most interesting to focus on a plant with potential to uptake relatively high amounts of heavy metals considering the acid mine drainage problem in this region. The family of *Salix* species, or willows, have been extensively studied in regards to their unique ability to transduce more heavy metals in comparison to other riparian species. In one study, *Salix exigua*, or coyote willow, appeared to have the highest capacity of four species to uptake, translocate and accumulate contaminants from a river (Brigmon, et al., 1999). Another study found that *Salix* species in general were much more likely to absorb heavy metals from the environment because they are fast growing, have better evapotranspirational abilities, and are deep-rooting phreatic trees, meaning they have the capability to reach contaminated groundwater.

In addition to this research, many studies are now focusing on the relationship between bacteria in the soil and the ability for willows to translocate more heavy metals than other plants in the same community. There is increasing evidence that rhizosphere bacteria may contribute to the metal extraction process in plants, although the mechanism behind this relationship is not well understood. This may, in part, explain why willows have a higher transduction ability, particularly with certain heavy metals such as cadmium and zinc (Kuffner, et al., 2008).

In regards to this evidence for heavy metal transduction, as well as the fact that *Salix* species are abundant in the San Juan region, coyote willow was the plant of interest of this study to better understand the riparian communities of the southwest, as well as their responses to unnaturally high heavy metal concentration. Implementing a heavy metal study on coyote willows in different zones and locations of the Animas River in response to the Gold King Mine spill allowed us to gather baseline data needed for determining the health of the river, and will serve as a template for future studies on abandoned mines and their long-term effects on plant and river ecology.

Since plants serve as bioindicators for entire ecosystems, quantifying their heavy metal uptake in a metal polluted area may help scientists reflect on what areas of the water table are under the most distress (Reay, 1972). Looking at three spatially unique riparian zones, and using multiple study sites helped us characterize the fate and transport of heavy metals, whether they are harming riparian plants, and what future responsibilities we have to contribute to the resiliency of the Animas river.

Methods

Study Sites

Three study sites were chosen for the collection of *S. exigua* within the San Juan watershed. The first two sites were on the Animas River, a 126-mile long river, which flows from headwaters at Silverton, Colorado, eventually converging with the San Juan River in

Northern New Mexico (Figure A). The first study site was located 5.3 miles south of Baker's Bridge, accessed via Trimble Lane, as the highest amounts of heavy metals were found close to Baker's Bridge post-mine spill. Based on limited presence of willows at the Baker's Bridge site, this was the closest accessible area with presence of *S. exigua.* The second study site was located 7.1 miles south of this site, at Oxbow Park and Preserve. This stretch of the river is characterized with meandering bends and less gradient than that of Baker's Bridge, attributing to the heavy sediment build-up at this potion of the river. Because these two reaches of the Animas River have unique geomorphology, they were chosen to observe the spatial differences in heavy metal concentrations on the Animas River.

The Florida River served as a control site, located at Durango Nature Studies, 12 miles south of Durango (Figure A). The Florida River is a 61-mile long tributary of the Animas River that is sourced from Lillie Lake in the Weminuche Wilderness. The Animas River had significant mining activity based in Silverton, Colorado, where the headwaters are found. In contrast, the Florida River has no past mining history. This made the Florida River an excellent control to add extra support when explaining how much of the metal concentrations in the Animas River are due to the mine spill versus natural environmental composition. Both rivers are heavily influenced by annual snowmelt higher up in the mountains during the spring and summer, as well as a monsoon season in the late summer to early fall (von Guerard, et al., 2004).

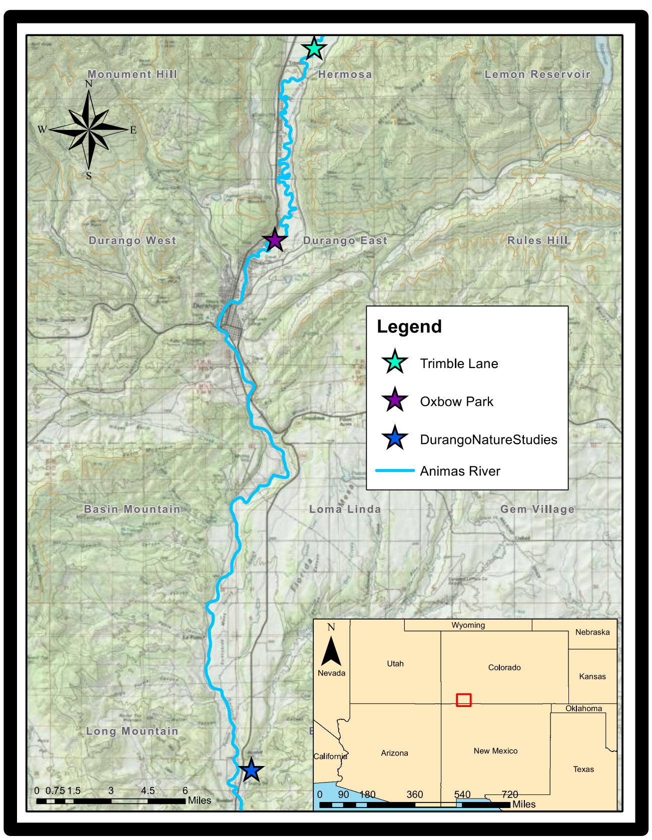


Figure A. Research study sites including Trimble Lane, Oxbow Park, and Durango Nature Studies.

Field Collection

The main focus of this study was to sample heavy metals in root and leaf samples of *S. exigua* to first see if there were any heavy metals present in these riparian plants. Furthermore, the question of interest was what parts of the plants had the highest concentrations amongst the different study sites and zones. At each study site, three zones were identified; the first zone was classified with young willow roots, in closest proximity to the river. Leaves of the younger willows composed the second zone, and mature, established willows made up the third zone, farthest from the river. This allowed us to observe how the willows were taking up heavy metals both temporally over their life span, and spatially in relation to the river.

Figure 1. The three zones chosen for field sample collection, with the first zone depicting the young willow roots, the second zone depicting young willow leaves, and the third zone depicting mature willow leaves. This image depicts one of the three study areas, at Oxbow Park and Preserve.

A preliminary test run was carried out before the main research study at Oxbow Park and Preserve to ensure that the willows had at least some form of heavy metal concentrations before complete sample collection. Two samples of the willow roots in the first zone and two samples in the second zone were collected and analyzed chemically using the MP-AES spectrometer before the initial study was thoroughly established. The preliminary test run concluded levels of six different heavy metals, so the full field collection proceeded.

Following the preliminary test runs, two 12- meter transects were set up with chaining pins at each site per zone, running parallel to the river. Four samples per zone were taken to ensure enough replication in the experiment. To account for a larger spatial distribution, and to avoid significantly damaging the willow population, three leaves or roots were clipped from each plant per meter. Each meter, a willow was sampled closest to the meter mark. This allowed for the study to to account for a larger proportion of the population size by taking leaves or roots from multiple willows. Root samples were stored in Ziploc bags and leaf samples were added to brown paper bags. All samples were labeled according to the location and sample number. This process was repeated once on each transect to account for more variability. The process was repeated for each zone by moving each transect south of the original transect. The distance moved was tentative to the area sampled, as some areas were limited by topography or amount of willows present. This method allowed for four samples per zone, and twelve overall per site. The distance and elevation between each zone was measured to account for the distance and vertical elevation differences.

After harvesting the willows, all samples were stored in the drying oven in brown paper bags for at least 48 hours to ensure there was no water content in the samples before chemical analysis was performed.

Chemical Analysis

i. Plant Digestion

After all plant samples were dried for at least 48 hours, root and leaf samples were transported to the analytical laboratory for the plant digestion process. Each site's samples were digested separately, with the addition of a standard reference material, or SRM. The SRM provided was a sample of NIST tomato leaves, which included known amounts of heavy metals, and allowed for the analysis method to be validated for plant matrices. Each sample was separately ground using a mortar and pestle, and 0.5 grams of each sample were weighed and transferred into a polypropyelene digestion vial. The sample number and biomass were recorded on each tube. From there, the digestion process proceeded, in which three reagents were all used in various dilutions to digest the samples: nitric acid, hydrogen peroxide, and hydrochloric acid. Following the addition of a reagent, the samples were heated on a ModBlock at 93 degrees C in different intervals depending on which reagent was added. After the digestion process, each sample was individually filtered with a 0.45 micron syringe filter apparatus into a volumetric flask and diluted to 50 mL with 1% nitric acid in distilled water (EPA Method 3050B).

Calibration standards were prepared at various concentrations (100, 200, 500, 700, and 1000 ppb) for each heavy metal being tested, through the sequential dilution of Agilent heavy metal standards. This allowed for the external calibration of the MP-AES instrumental response to a range of heavy metal concentrations.

ii. Atomic Emission Analysis

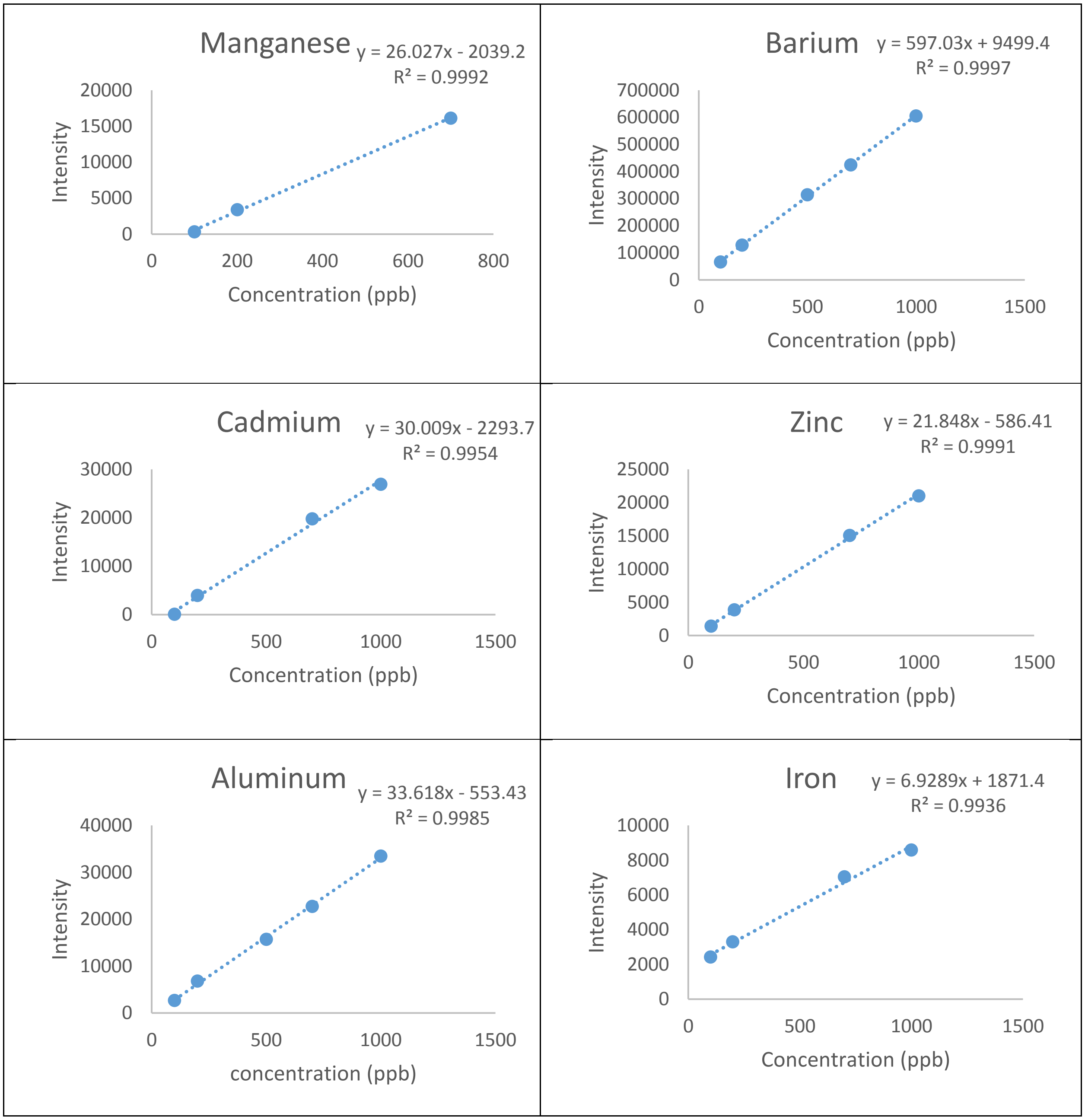
Following the acid digestion of plant samples, which solubilized the heavy metals, the samples were ready to be analyzed with the MP-AES 4200 instrument. Each sample was separately analyzed by plastic tubing that would automatically uptake each individual sample. A blank solution of 1% nitric acid in distilled water was tested in between any samples to rinse the apparatus to avoid cross-contamination in between samples.

The MP-AES ran almost entirely on compressed air, coupled with a nitrogen generator, which removed all oxygen from the machine. The nitrogen allowed for a plasma that generated a high intensity emission line. Samples were passed through a nebulizer which vaporized each sample. From there, they were passed through a high heat plasma, which atomized the sample, allowing the heavy metals of the sample to emit photons at predictable wavelengths and measurable intensities. Photons with wavelengths characteristic for each element of interest were then measured by a CCD detector (Agilent Technologies, 2014). Each sample was tested by the instrument ten times per trial, increasing reliability of the results. The samples were tested for six heavy metals: zinc, manganese, iron, cadmium, aluminum, and barium.

Data Analysis

Data analysis was performed using Microsoft Office Excel after all heavy metal concentrations were recorded. Data was exported from the MP-AES 4200 software into Microsoft Excel, and actual concentrations and standard deviations were calculated. A 2-way ANOVA test was performed using SPSS to account for any statistically significant data between sites, zones, as well as site-zone interactions. A p-value of 0.10 was used to conduct analyses as variation is typically higher in a natural setting. In addition to this, high variability was observed between individual samples, as the metals are typically parts per billion, so even a small change can have a large effect on these small values.

Results



Figures 1(a-f). Calibration curves for all six heavy metals that were analyzed. Calibration solutions were prepared for 100, 200, 500, 700, 1000 ppb, respectively using known amounts of Agilent heavy metals that were diluted using 1% nitric acid in deionized water.

Before any of the experimental data was analyzed, the calibration standards were plotted to ensure a high R-squared value. This reassured us that the MP-AES 4200 was properly calibrated. All six heavy metals at this point had an R-squared value of over 99%, which allowed us to move on to the experimental data. The MP-AES instrument took measurements in parts per billion, which were then translated into mg/kg taking both the original dilution factor (0.05 L), as well as the mass per each sample, into account.

Table 1. Standard reference material corrected concentration values. These values were applied to experimental plant data to correct for instrumental error. The average percent errors were used to determine if the heavy metals in experimental samples should be analyzed.

Element	Actual Value	Experimental Value	Average % Error
Al	598	206.7	-45.3
Cd	1.5	11.8	-1.5
Ba	63	15.8	-12.0
Zn	30.9	81.5	-1.6
Fe	368	34.2	-0.2
Mn	246	133.6	-1.2

A correction factor was made for each plant sample as standard reference materials were digested with the experimental samples. The correction factor was determined based upon the known amounts of heavy metals in the standard reference material compared to the experimental results from the standard reference material. As the percent errors for aluminum and barium were fairly high, the experimental data for these two elements were not used to assess the heavy metal concentrations of plants on the Animas River.

Aluminum concentrations far exceeded the calibration standards prepared, as the standards could only accurately measure concentrations from 100 parts per billion to 1000 parts per billion. On the other hand, barium was also excluded from data analyses as almost all concentrations were below 100 parts per billion, of which no calibration standards were prepared.

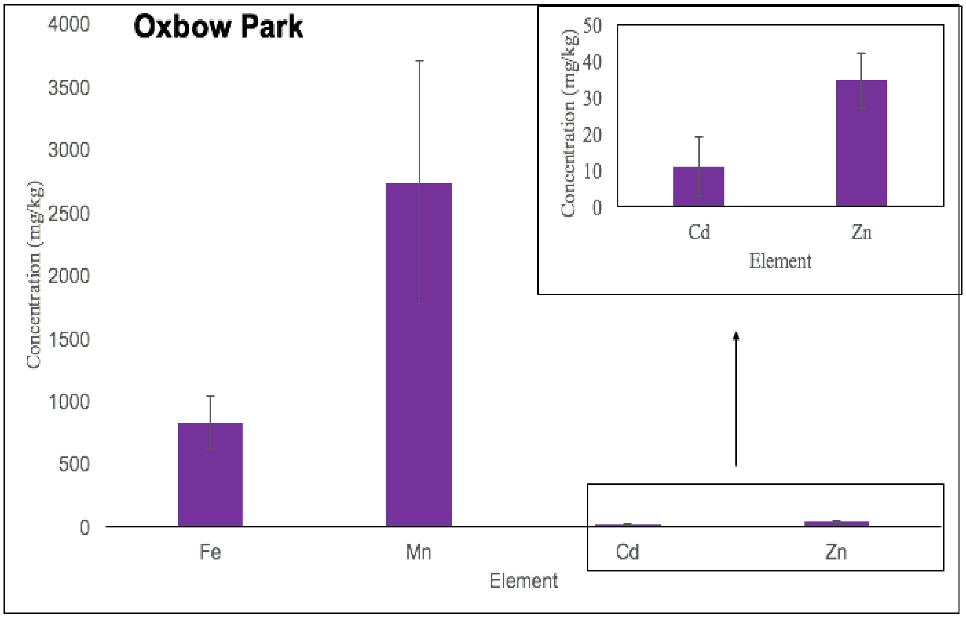


Figure 2. Average concentrations of the four observed heavy metals at Oxbow Park and Preserve, one of the treatment sites.

Oxbow Park and Preserve had the highest overall concentrations of heavy metals, compared to Trimble Lane and the control site, Durango Nature Studies on the Florida River. Figure 2 helps exemplify the amounts of each metal relative to one another at the same site. Averages of metals were taken for each of the twelve samples. It is apparent that all concentrations are higher at Oxbow Park versus the control group, and that all concentrations are highest at Oxbow Park, followed by Trimble Lane.

The values for cadmium and zinc were displayed separately so that the error bars and concentrations could be clearly seen.

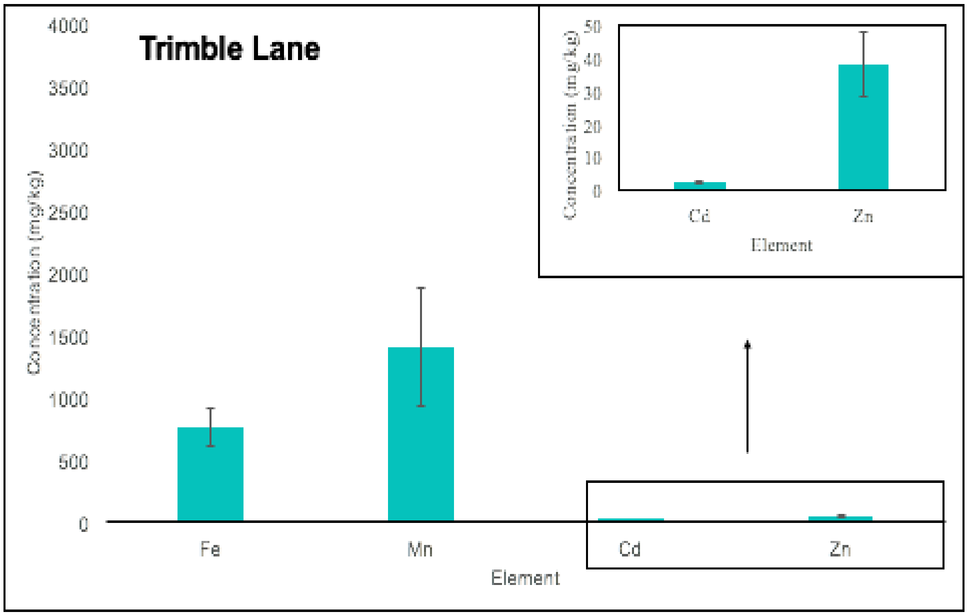


Figure 3. Average concentrations of heavy metals at the Trimble Lane site, the second treatment site.

Again, the chemical analyses did show heavy metals for each of the four heavy metals tested, all in very different quantities. Trimble Lane had higher concentrations for all heavy metals than that of the control site; however, all averages at Trimble Lane were lower than at Oxbow Park and Preserve, which may reflect the different geomorphology and speed of the river.

Similar to Figure 2, cadmium and zinc were displayed on a separate graph so that their concentrations and error bars could be more visually apparent.

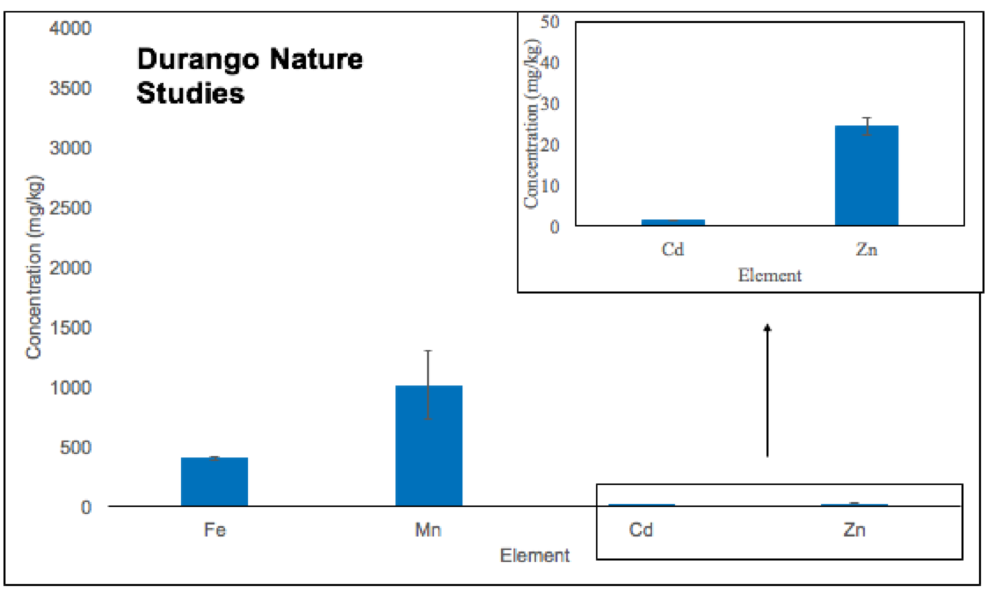


Figure 4. Average concentrations of heavy metals at the control site, Durango Nature Studies on the Florida River.

Because cadmium and zinc concentrations were exceptionally lower than iron and manganese, they are displayed above on a separate graph for clarity of average concentrations and error bars. While there is less variation in metal loads at this sample site, it is quite clear than the concentrations are much lower at the control site than at Trimble Lane or Oxbow Park, which was expected based on the fact that there is no current or past mining history on this river.

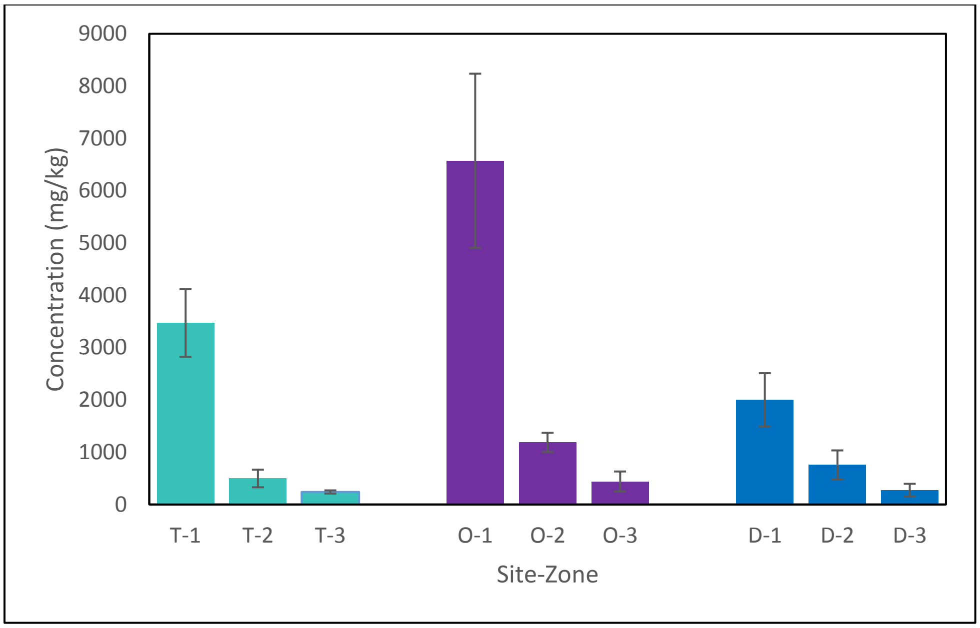


Figure 5. Average concentrations of manganese at the three sites, and separated out into the three different zones. T indicates the first site at Trimble Lane, O indicates the second site at Oxbow Park and Preserve, and D indicates the control site at Durango Nature Studies.

The figure depicts a trend present across all study sites, with the highest concentrations of manganese in the roots, second highest in the young leaves (zone 2), and least in zone three (mature leaves). However, zone 1 did have the highest error bars across all sites, indicating higher variability amongst the root samples, where some had very high amounts of manganese and some individual samples contained fairly low amounts.

For consideration of the interactions between all sites and zones, there were significantly different amounts of heavy metals, with a p-value of 0.041; however, different statistical analyses were observed for specific comparisons between zones and sites.

A two-way ANOVA test was used to assess significance between all three sites, as well as all three zones. There was no significance for manganese concentrations between Trimble and the control group Durango Nature Studies (p-value=0.792). However, there was a significant difference between the amounts of manganese found between Oxbow and the control site(p-value=0.021), as well as Oxbow and Trimble (p-value=0.087).

The two-way ANOVA test was also used to compare samples between zones as well. There was no significance between zones 2 and 3 (young plants and mature plants, respectively), with a p-value at 0.689. However, all other zones did show significantly different amounts of manganese. Zones 1 and 2 (roots and young leaves, respectively) had a p-value of less than 0.001, as did the roots and old leaves, which accounted for zones 1 and 3.

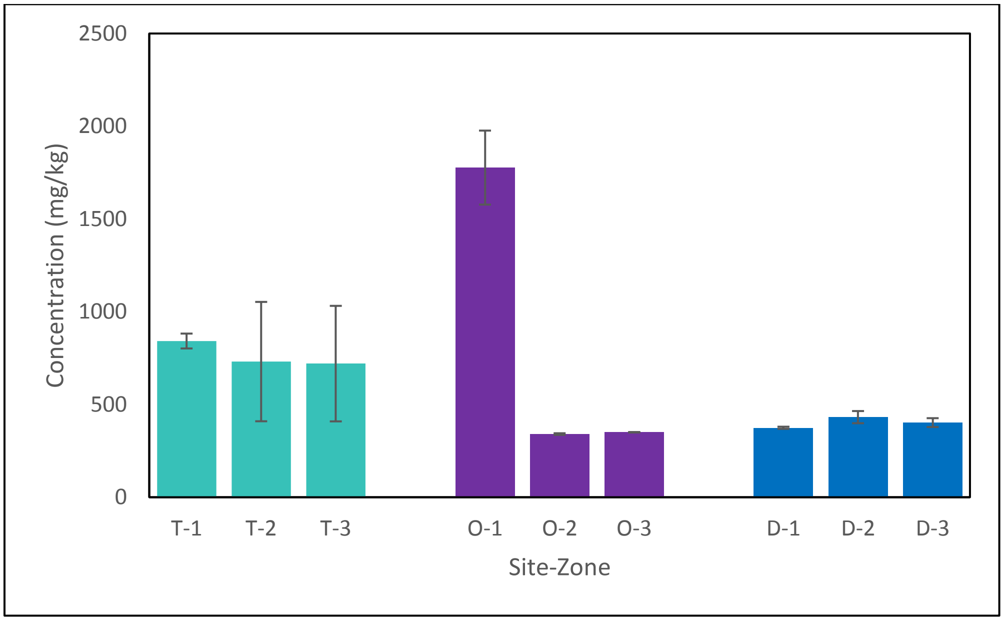


Figure 6. Average concentrations of iron found in willow species at the three study sites. T indicates the Trimble Lane study area, O indicates the Oxbow site, and D indicates the Durango Nature Studies area.

There tended to be less variability in terms of error bars; however, the data is not as consistent across sites, or across zones. Where the Oxbow and Trimble sites show declining iron concentrations as you proceed away from the river, the DNS site exemplifies zone 2 with the highest iron concentrations.

Figure 5 illustrates distinct differences between all sites, zones, and the interactions between the two. This was exemplified with a 2-way ANOVA test, which yielded a p-value of 0.001 for site and zone interactions. The sites and zones were also analyzed individually, which again, displayed different results.

Iron showed fairly similar trends to manganese in terms of significant data. There was no significant difference in iron concentrations between Oxbow and Trimble sites, with a p-value of 0.926. In contrast, there was an overall observable difference between Trimble and the control site, Durango Nature Studies, yielding a p-value of 0.068. Furthermore, Oxbow and the control site, DNS, were significantly different with a p-value of 0.030.

In terms of zones, there was no significance between the young and old leaves (zones 2 and 3), with a p-value of 0.998. However, there was a significant difference in amounts of iron between zones 1 and 2, yielding a p-value of 0.009. Similarly, zones 1 and 3 displayed significance with a p-value of 0.008.

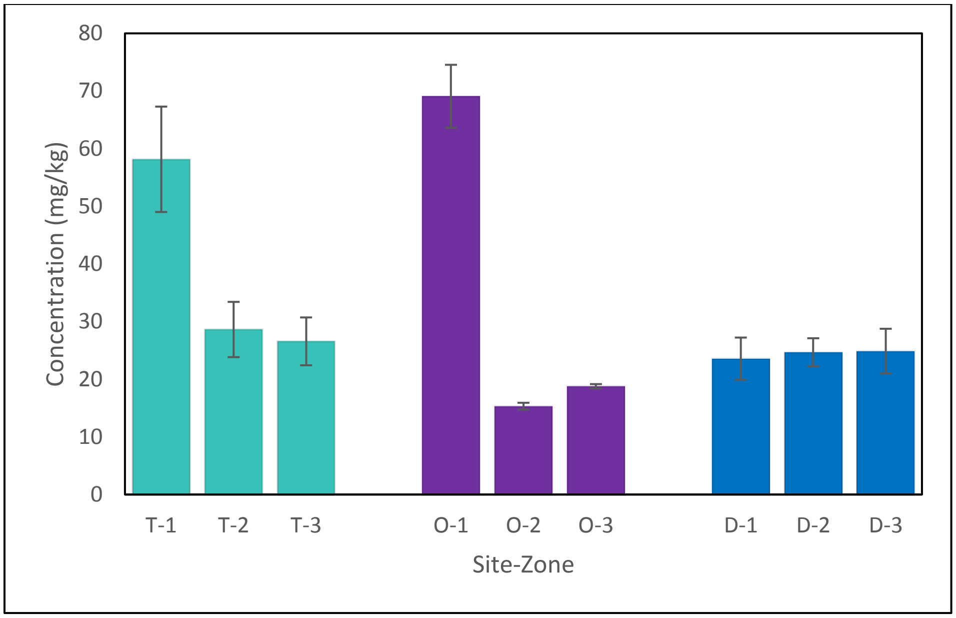


Figure 7. Average concentrations of zinc at the three study sites, divided into the three ecological zones.

There was a wide array of trends exhibited across the three zones and sites for concentrations of zinc. Trimble showed the most common trend, with heavy metal load decreasing as you move away from zone 1. The Oxbow site exhibited very high concentrations at zone 1, with zones 2 and 3 much lower. The control site displayed the most evenly distributed metal loads across all three sites.

Figure 7 demonstrated significantly different amount of zinc concentrations in terms of the interactions between sites and zones. This was tested using a 2-way ANOVA, which gave a p-value of 0.000, meaning all site and zone interactions were different. Site and zones were then analyzed individually to look closer at variation between the three sites, and the three zones separately.

There were no statistically significant differences found between the Oxbow and Trimble sites, with a p-value at 0.743. The sites that varied significantly in their zinc load were the Trimble site and the control site, with a p-value of 0.012, as well as Oxbow and the control site, with a p-value of 0.063.

In regards to the differences in zinc across the three zones, there was no significance between young leaves and old leaves, with a p-value of 0.984. Like the other heavy metals, there was a significant difference in zinc between zones 1 and 2, with a p-value less than 0.001. The difference in zinc between zones 1 and 3 was also significant, yielding a p-value of less than 0.001.

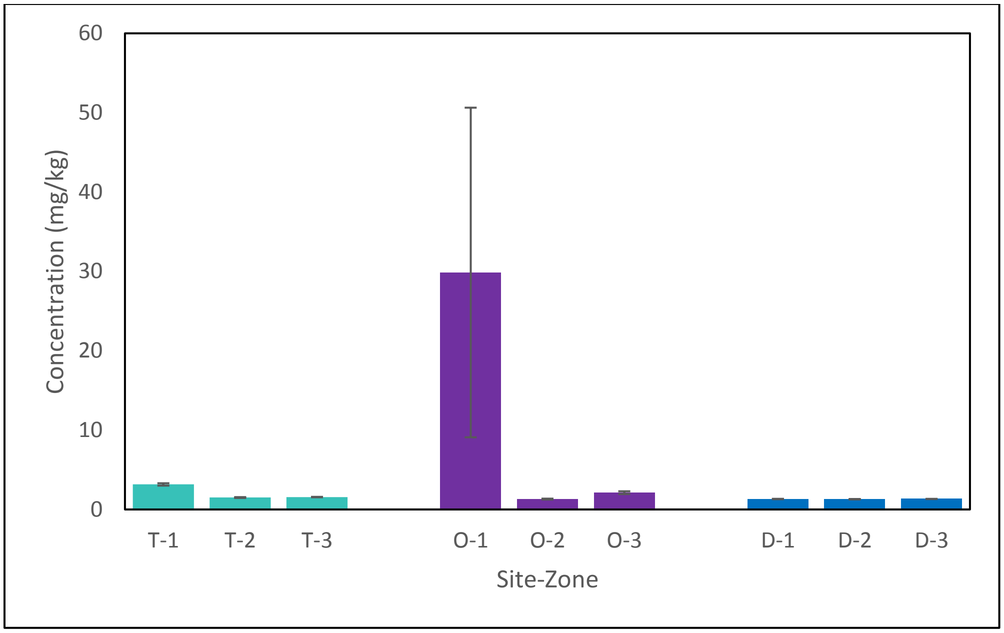


Figure 8. Average concentrations of cadmium across the two treatment sites, and one control site. Each treatment or control is subdivided into the three zones, containing roots, young leaves, and mature leaves.

Compared to the other metals found in the plant samples, cadmium was present in the lowest concentrations across each site. The root samples at Oxbow were significantly greater than any other zone or site; however, they also had extremely high error, as one root sample had very high levels of cadmium compared to the other samples.

Interestingly, cadmium was the only heavy metal that did not exhibit any significance for site and zone interactions. The p-value came out at 0.294, by far the highest of any of the heavy metals.

Cadmium was the only metal that did not exhibit any significance across study sites, Oxbow and Trimble yielding a p-value of 0.364, Trimble and the control yielding a p-value of 0.993, and Oxbow and the control displaying a p-value of 0.307.

The same trend was observed for cadmium across the three zones. The roots and young leaves (zones 1 and 2), showed a p-value of 0.287, and the roots and old leaves (zones 1 and 3) had a p-value of 0.308. There was no significance, as expected, between the young and old leaves, with a p-value of 0.999.

Discussion

Following analyses of the results, the main conclusion was that *S. exigua* has a wide variation of heavy metal accumulation, in terms of site location, whether the samples are roots or shoots, and which heavy metal is present. Having three site locations allowed for comparison of not only the Animas River with a control river, but also comparing various stream morphologies. As Oxbow Park has a slower river flow and consists of meandering characteristics, there is more sediment deposition onto the point bars. After the plume, the high concentrations of heavy metals were most likely deposited in higher levels than at other places. This is reflected by water quality tests that were conducted right after the Gold King Mine spill, which showed highest levels of contaminants at Baker's Bridge, followed by Oxbow Park (Steltzer, 2015). Oxbow Park was always significantly different in metal loads from the control group, while Trimble Lane was not always different from the control. Again, this may be due to the fast moving water and anabranching features that make up the area around the Trimble site.

Looking deeper into this study, the most apparent trend observed was the significantly higher concentrations of all heavy metals in the roots, compared to the leaves. This was evident, regardless of which site the plants were located at, or which metal was being tested for. When compared using a 2-way ANOVA, the differences in concentrations between the root samples (zone 1) and the leaf samples (zones 2 and 3), were almost always statistically significantly different. The only metal tested that did not show significantly different concentrations between roots and leaves was cadmium, which may be due to the relatively low concentrations observed in the plants. The overall trend of higher metal concentrations observed in the roots can be supported by past data, particularly *Salix spp.* Since roots are the first part of a plant to come into contact with the metal ions, and transport of the heavy metals to shoots is relatively low, they typically accumulate higher concentrations stored in the roots (Landberg & Greger, 1994). This is most likely a mechanism evolved to protect the shoot, and therefore, the photosynthetic pathways of the plant from being affected (Landberg & Greger, 1996). There were never significant differences between the young plant leaves and the mature plant leaves, which may mean that there is not a lot of transduction occurring from the roots to the shoots, even over the long term.

Another interesting trend observed in this study was the variation of concentrations between metals. Clearly, the metals were all taken up at different levels, as the concentrations vary in the water itself. Aluminum and manganese are much higher in the water, which makes sense that these were the two highest metals observed (USDI, 2015). Furthermore, metals are taken up in different amounts depending on both the tolerance and nutrient needs of the plant. Metals such as zinc and manganese are needed as secondary metabolites, so willows tend to uptake higher quantities of these metals. In addition, *S. exigua* has been noted to have a high tolerance for metals such as cadmium and zinc, which may be due to their symbiotic relationship with rhizosphere bacteria which has a high affinity for these metals, and allows for better extraction of these metals from the sediment into the roots (Kuffner, et al., 2008).

Future studies would be beneficial to getting more data, and thus, more evidence of elevated heavy metals on the Animas River. Because there were only twelve samples per site and four samples per zone, the variation in heavy metal concentrations was high, even within one site or zone. For a similar study in the future, perhaps eliminating one variable may help increase the replication. By just looking at roots versus leaves, or looking at two sites instead of three, more samples could be taken to reduce the variation that was observed in this study. It would also be of interest to take samples from the same site over the next decade, in order to see if any transduction is occurring from the roots to the shoots. Particularly in regards to phytoremediaton efforts, if the roots are transporting heavy metals to the leaves in high quantities, *S. exigua* could be a potential natural resource for remediation efforts. As stated earlier, the efforts that exist now are simply prevention techniques, as well as chemical treatment of the water to raise the pH.

The plants on the Animas River are clearly characterizing higher heavy metals concentrations than on similar rivers in the surrounding area. This reflects that anthropogenic effects are being not only causing poor water quality; the effects are being amplified into the riparian zones, and potentially the entire ecosystem in this area. While heavy metals are more prevalent in this area considering the environmental variables, such as the type of rock and minerals, we need to make a conscious effort to reduce the amount of metal pollutants that are being excreted into water sources.

Works Cited

Agilent Technologies. 2014 “Agilent 4200 microwave plasma-atomic emission spectrometer: change is in the air”. *www.agilent.com*

Ahluwalia, S.S., and Goyal, D. 2007. “Microbial and plant derived biomass for removal of heavy metals from wastewater”. *ScienceDirect*. *(98), 2243-2257.*

Becker, Hank. 2000. “Phytoremediaton: using plants to clean up soils”. *Agricultural Research 48(6), 4-9.*

Cutright, T.J., Senko, J., Sivaram, S., & York, M. 2012. “Evaluation of the phytoextraction potential at an acid-mine-drainage-impacted site”. *Soil and Sediment Contamination, 21(8), 970-984.*

Gray, N.F. 1997. “Environmental impact and remediation of acid mine drainage: a management problem”. *Envrionmental Geology*. *30(1), 62-71*.

Johnson, D. B., & Hallberg, K.B. 2005. “Acid mine drainage remediation options: a review”. *Science of the total environment*, *338*(1), 3-14.

Kuffner, M., Puschenreiter, M., Wieshammer G., Gorfer, M., and Sessitsch, A. 2008. “Rhizosphere bacteria affect growth and metal uptake of heavy metal accumulating willows.” *Plant Soil, 304: 35-44.*

Landberg, T & Greger, M. (1996). “Differences in uptake and tolerance to heavy metals in *Salix* from unpolluted and polluted areas”. *Applied Geochemistry 11: 175-180.*

Mleczek, M., Kozlowska M., Kaczmarek, Z., Magdziak Z., and Golinski, P. 2011. “Cadmium and lead uptake by *salix viminalis* under modified ca/mg ratio”. *Ecotoxicology 20: 158-165.*

Mourato, M.P., Moreira, I.N., Leitae, I., Pinto, F.R., Sales, J.R., and Martins, L.L. 2015. “Effect of heavy metals in plants of the genus brassica”. *International Journal of Molecular Sciences, 16(8), 17975-17998.*

Rai, P.K. 2008. “Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an ecosustainable approach”. *International Journal of Phytoremediation, 10(2): 133-160.*

Reay, P.F. 1972. “The accumulation of arsenic from arsenic-rich natural waters by aquatic plants”. *Journal of Applied Technology.*

Soltan, M.E., Koraiem, A.I., Ahmed, M.M., and Mahfouz, M.A. 2009. “Distribution and accumulation of heavy metals in the aquatic plants at northern egyptian lakes”. *Rural Development 59-68.*

Steltzer, H. 2015. “River forum summary: fate and transport of metals in the animas river”. *Fort Lewis College Biology Department.*

Tangahu, B.V., et al. 2011. “A review on heavy metals (as, pb, and hg) uptake by plants through phytoremediation”. *International Journal of Chemical Engineering.*

USDI. 2015. “Technical evaluation of the gold king mine spill”. *U.S. Department of the Interior.*

Von Guerard, P., Church, S.E., Yager, D.B, AND Finger, S.E. 2004. “Integrated Investigations of environmental effects of historical mining in the animas river watershed, san juan county, colorado”. *USGS.*